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Investigating the algae for the synthesis of biofuel by using genetic engineering techniques

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Abstract

Fossil fuels are the main energy source despite they are not producing greenhouse emissions in environment. Research into microalgae genomics resulted in multiple genome sequences where Chlamydomonas reinhardtii received attention because scientists could utilize effective genetic tools. Scientists are developing testing methods for diatoms and industrial algae species but also other significant industrial microorganisms. Scientists worldwide can conduct genetic transformation on all divisional algal species since they successfully adapted more than 30 strains. The insertion of random transgenes through antibiotic markers is possible with nuclear transformation but experts face difficulty in targeted gene deletion due to limited homologous recombination success rates.

Introduction

All human beings need energy to survive daily life together with other living organisms. Major energy consumption occurs when people use heat sources for basic needs and cooking food and operate electrical systems and transportation networks. Their life depends heavily on energy for all living beings along with humans. The basic functions of heating cooking and transportation with electricity demand high amounts of energy usage. The primary energy source of modern times continues to be fossil fuels because we need these fuels extensively [1]. Fossil fuels originate from buried biomass through natural geological processes that took place during millions of years. The three major fossil fuel groups include coal as an element alongside oil followed by natural gas [2]. Atmospheric release of fossil fuels leads to substantial greenhouse gas emissions as their main environmental challenge. The carbon dioxide (CO_2) and methane (CH_4) along with sulfur oxides (SOx) and nitrogen oxides (NOx) greenhouse gases escape the atmosphere during every step of fossil fuel production from extraction to energy consumption. Human-produced gases from industrial activities create global warming and subsequently cause both climate change disorder and ocean acidification and environmental contamination. It is pulmonary disease that humans develop because of sulphur oxide pollutants along with the soot particles created by fossil fuel combustion. Human health risks from hydraulic fracturing chemicals include water contamination that leads to cancer development in humans. Using fossil fuels leads to multiple adverse results such as degraded soil quality and ecosystem destruction and environmental contamination caused by oil spills and gas leaks in the environment [3]. The level of atmospheric CO_2 increased from 280 ppm to 419 ppm throughout the period from 1900 to 2019 according to available data [4]. Biofuel stands as a principal alternative to fossil fuels because it releases less greenhouse gases including CO_2 and serves the transportation sector best [5]. Biofuels exist as first second third and fourth generation products according to the specific source materials utilized [6]. Sugar cane biofuel production initiated in Brazil when the country became the first country to harness this raw material for their fuel industry [7]. Biofuels may produce feedstocks from waste and residual non-food-related biomass to create biofuels which include waste vegetable oil and agricultural residue (i.e. lignocellulosic biomass [8]. According to the feedstock type biofuels exist in four generations: first-, second-, third and fourth-generation biofuels [9]. First-generation biofuels develop from fermentation processes using sugar cane and vegetable oils as food crops [10]. Through sugar cane fermentation Brazil established its initial biofuel raw materials program to create bioethanol fuel from sucrose-rich juice [7]. Algae serve as the leading material for producing third and fourth-generation

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biofuel technologies with microalgae being the optimal feedstock selection [12]. Algal-based biofuel production shows its principal differentiating factor in utilizing algae genetic modification between third and fourth generation biofuel processes [13]. A 28% rise in biofuel market demand is projected from 2021 through 2026 to reach between 41 to 53 billion liters according to forecast reports [14]. These biofuels have their fundamental chemical and physical attributes examined against traditional fuel sources in Table 1.

	Biogas	Bioethanol	Biobutanol	Biodiesel	Gasoline
Number of carbon atoms	1	2	4	12–20	4–12
Density [kg L^{-1}]	0.00115[20]	0.79[21]	0.81[21]	0.88[22]	0.74[22]
Viscosity at 20°C [mm^2 s^{-1}]	-	0.5[23]	3.3[24]	5.1[23]–7.5[22]	0.6[22]
Octane number	-	>100[22]	87[23]	-	92[22]
Cetane number	-	8[22]	17[23]	56[23] [22]	15–20
Lower heating value [MJ kg^{-1}]	23.3[25]	27[22,23]	36[23]	37[23] [22]	43.9[22]
Heating value [MJ L^{-1}]	0.016–0.028 [25]	21.06[22]	29.00[26]	32.65[22]	32.48[22]
Flash point [°C]	-	13[23]	35[23]	160[23]/120	<21[22]
Fuel equivalence [L]	-	0.65[22]	-	0.91[22]	1[22]
Estimated price per heating value [Euro MJ^{-1}]	0.03[27]	0.07[28]	0.06‡ [29]	0.05[30]	0.05[31]

‡ As biobutanol has not achieved significant market penetration, estimated price is based on conventional n-butanol. Biobutanol prices should exceed conventional prices, given the extensive and energy demanding purification process.

First-generation biofuels

Biofuels from the first generation consist mainly of bioethanol together with biodiesel. The first-generation bioethanol industry relies on microbial fermentation of edible material containing starch and sucrose which includes wheat and corn in Europe and North America as well as sugarcane in South America. The commercial strain groups consist of Saccharomyces cerevisiae together with S. stipites and S. pombe. Bioethanol production extends beyond first-generation biofuels according to the feedstock and production strain classification [15-16].

Second-generation biofuels

Second-generation biofuels emerged because first-generation biofuels were met with problems through the deployment of lignocellulosic biomass which originates from agricultural residues and woodland remnants and waste streams coming from food-related operations such as wheat bran and cooking and frying oil disposals. Added to the potential biofuel sources for the second generation stands the drought-tolerant Jatropha curcas shrub or tree which survives in wasteland areas [17].

Third-generation biofuels

Microalgae and cyanobacteria biomass serve as the main source for third-generation biofuels through natural alcohol and lipid generation that allows production of biodiesel and any high-energy fuel products. Photosynthesis by algae produces biomass at twice to four times higher rates than terrestrial plants [18]. The biological cultivation of algae occurs without needing either land-based fields or fresh water supply resources. The use of waste water and brackish or salt water for growing cultures proves to be cost advantageous and eliminates agricultural competition [19,20]. A greater quantity of oil can be produced using algae since lipids tend to accumulate in specific plant areas whereas algae cells contain abundant lipids making the production process more cost-efficient [21].

Fourth-generation biofuels

The latest biofuel generation known as fourth-generation biofuels incorporates genetic engineering to modify organisms used in biofuel production through genetic engineering techniques. When scientists modify algae genetically the result is mostly improved output production and additional advantageous attributes as opposed to natural algae. Scientists transfer the antennae systems from algae with wide light spectrum absorption capabilities to production organisms that need enhancement [22,23]. The genetic engineering tool CRIPSR/Cas9 finds extensive use because it provides an easy design procedure and achieves effective transfection combined with targeted gene disruption [24]. Research conducted in Europe determined most customers would welcome the use of genetically modified algae as biofuel raw materials but only under conditions of proven system safety. When containment systems operate correctly and selection processes choose appropriate locations the associated safety risks can dramatically decrease. Benefits with high safety standards will drive the construction of these closed production systems [25]. Biocontainment features derived from genetic modifications inside production cells that include auxotrophies and kill switches help decrease genetically modified organism (GMO) escape risks substantially [26-27].

Materials and methodology

Genetic engineering of microalgae

Genomic research on microalgae has experienced major advancements since the last ten years. Professionals have developed expressed sequence tag (EST) databases and they have sequenced multiple microalgal nuclear genomes as well as mitochondrial and chloroplast genomes. Several new microalgal genome sequences are underway. Most molecular and genetic phycological research concentrates on the green alga Chlamydomonas reinhardtii as its primary subject. Most available technologies designed to express transgenes and perform gene knockdown operate only within this particular species. Scientists have recently launched quick development of analytic tools for diatoms along with other algae groups considered significant for industrial production.

Microalgal genomes

Multiple studies have finished sequencing nuclear genomes with C. reinhardtii [28-29] as one of the successful projects. Phaeodactylum tricornutum[30],Ostreococcus lucimarinus [31]. Thalassiosira pseudonana[32]. Cyanidioschyzon merolae [33]. and Micromonas pusilla[34]. The list of active microalgal genome sequencing projects includes analyses of Fragilariopsis cylindrus, Pseudo-nitzschia, Thalassiosira rotula, Botryococcus braunii, Chlorella vulgaris, Dunaliella salina, Micromonas pusilla, Galdieria sulphuraria, Porphyra purpurea, Volvox carteri andAureococcus anophageferrens [35].

Multiple initiatives involving the sequencing of plastid and mitochondrial genomes and dynamic transcriptomic studies exist for different microalgae species both past and present [41].

Methods for transformation and expression

The scientific field of genetic transformation has become possible for all algae divisions including Chlorophyta as well as Rhodophyta and Phaeophyta and diatoms and euglenids and dinoflagellates [36]. Researchers successfully transformed more than 30 different strains of microalgae at present. The genetic transformation methods allowed the establishment of transgenes through stable expression from nuclear or plastid elements but some methods led to temporary transgene expression. Science based on genetic transformation of C. reinhardtii forms the main body of microalgal research which Eichler-Stahlberg et al. reviewed in their article 37. Biolistic microparticle bombardment [40] joins glass bead agitation and electroporation [39] among other methods like agitation with silicon carbide whiskers or glass beads [38] as transformation techniques for microalgae DNA delivery. The selection of transformants among microalgae relies on various antibiotic resistance genes which have shown success in bleomycin selection [41]. Paromomycin [42]. nourseothricin [43]. Chloramphenicol [44] and others. The random insertion of transgenes occurs throughout the nuclear transformation process of microalgae. The capability to expresss transgenes through random mutagenesis works well but prevents targeted deletion of particular genes. Scientists have achieved limited advancements in homologous recombination when working with the Leiper and Gobel carry out gene editing operations on C. reinhardtii nuclear genome but the method yields low success rates [46]. The report verifies that homologous recombination takes place in the red form of microalga C. merolae [49]. Artificial-micro-RNA (armiRNA) techniques for C. reinhardtii provide high-throughput gene knockdown methods that researchers now use because the approaches show better specificity and generate more stable effects than traditional RNA interference (RNAi) methods [50]. The actual number of antibiotics which function successfully against a specific strain becomes very restricted because numerous microalgae show widespread resistance toward various antibiotics. Using salt-based media makes nourseothricin and G418 antibiotics ineffective which makes them unsuitable for marine algae applications [47]. Biofuel production requires correct expression and localization of transgenes in chloroplasts in order for many metabolic genes to function properly. Transformation of the chloroplast in C. reinhardtii becomes achievable through homologous recombination as explained in the review by [48].

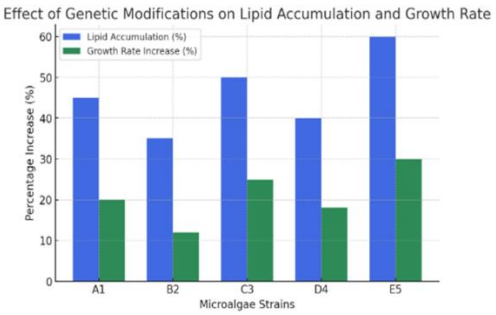
Genetic engineering of the lipid metabolism

Understanding microalgal lipid metabolism is of great interest for the ultimate production of diesel fuel surrogates. Both the quantity and the quality of diesel precursors from a specific strain are closely linked to how lipid metabolism is controlled. Lipid biosynthesis and catabolism, as well as pathways that modify the length and saturation of fatty acids, have not been as thoroughly investigated for algae as they have for terrestrial plants. However, many of the genes involved in lipid metabolism in terrestrial plants have homologs in the sequenced microalgal genomes. Therefore, it is probable that at least some of the transgenic strategies that have been used to modify the lipid content in higher plants will also be effective with microalgae. Hazard assessments

The initial phase within hazard assessment requires identification of negative consequences that originate from GM algae organisms or those from their transferred traits. Green algae remain environmentally benign except the health threat from Prototheca wickerhami that causes Protothecosis disease in dogs along with cats, cattle and humans. The organism lacks sufficient value for serving as a biofuel production source. Cyanobacteria create toxins and multiply rapidly at welcoming environmental conditions to form toxic algae blooms that result in major worldwide ecological and human health issues. The presence of blooming cyanobacteria results in toxic cyanotoxin production that threatens populations of fishes as well as animals and humans. The toxins produced by cyanobacteria will concentrate within fish and shellfish after which they make their way into human food. Neurotoxins and hepatotoxins along with cytotoxins and endotoxins comprise common cyanobacterial toxins that endanger health of humans and animals and marine organisms. The commercial production of biofuels from cyanobacteria remains without identified specific candidates at present. Only model species make up the majority of genetically modified strains that scientists have developed until this point.

Exposure assessment

Commercial production of GM algae poses the risk of discharging organisms into natural environments. A detailed exposure assessment requires thorough evaluation of the possible pathways through which organisms would contact their natural environment. The development of Digital PCR technology aims to provide specific detection abilities for identifying GM algal strains within surrounding environments. Since GM algae production remains absent from commercial-scale open cultivation operations a comprehensive exposure assessment remains impossible to achieve leaving only theoretical generalizations available for exposure assessment. The influence of lipid metabolism controlling genes incorporated in construction elements for algal transformation procedures is assessed through comparison of multiple methods. Strains of microalgae that show increased lipid accumulation capability were obtained through genetic engineering methods which also enhanced lipid composition along with algal growth performance.



Conclusions

Researchers have successfully developed genetic tools for microalgae which focus mainly on homologous recombination and gene knockdown approaches. The scientific community achieved homologous recombination success with C. merolae which permits more genetic modifications across many microalgal species. The invention of high-throughput artificial-micro-RNA (armiRNA) methods for C. reinhardtii serves as a more precise and stable system than conventional RNA interference (RNAi) while raising gene silencing performance. The main issue in microalgal genetic engineering involves the resistance of microalgae to antibiotics because most of these microorganisms do not respond to common antibiotics. There is a scarcity of effective antibiotics to select and maintain genetically modified strains particularly in salt-containing media which causes nourseothricin and G418 to become ineffective at selecting modified strains especially within marine algae.

Transgene expression along with controlling protein localization inside chloroplasts ensures proper metabolic functionality ofgenes which mainly focus on biofuel production. Scientists use homologous recombination to

modify genes inside chloroplasts of C. reinhardtii cells. Current developments prove microalgal genetic engineering has great potential for biofuel production and biotechnological applications yet demonstrate the necessity for more research to solve current obstacles especially related to antibiotic selection and marine algal gene editing.

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